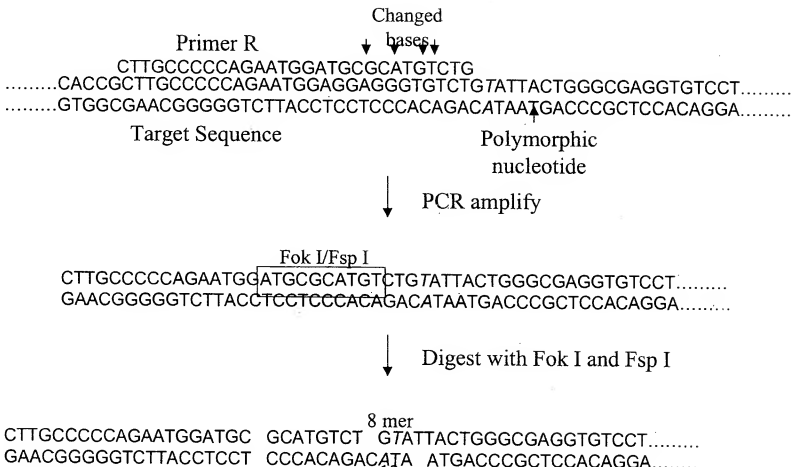

Changed
bases
Primer R
CTTGCCCCCAGAATGGATGCGCATGTCTG
.....CACCGCTTGCCCCCAGAATGGAGGAGGGTGTCTGTATTACTGGGCGAGGTGTCCT.....
.....GTGGCGAACGGGGGTCTTACCTCCTCCCACAGACATAATGACCCGCTCCACAGGA.....
Target Sequence
Polymorphic
nucleotide
PCR amplify
Fok I/Fsp I
CTTGCCCCCAGAATGGATGCGCATGTCTGTATTACTGGGCGAGGTGTCCT.....
GAACGGGGGTCTTACCTCCTCCCACAGACATAATGACCCGCTCCACAGGA.....
Digest with Fok I and Fsp I
8 mer
CTTGCCCCCAGAATGGATGC GCATGTCT GTATTACTGGGCGAGGTGTCCT.....
GAACGGGGGTCTTACCTCCT CCCACAGACATA ATGACCCGCTCCACAGGA.....
12 mer

FIG. 1

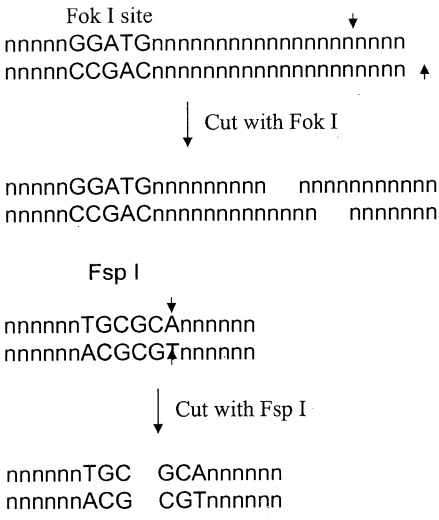

Fok I site
nnnnnGGATGnnnnnnnnnnnnnnnnnnnn
nnnnnCCGACnnnnnnnnnnnnnnnnnnnn
Cut with Fok I
nnnnnGGATGnnnnnnnnn nnnnnnnnnnn
nnnnnCCGACnnnnnnnnnnnnn nnnnnnn
Fsp I
nnnnnnTGCGCAnnnnnnn
nnnnnnACGCGTnnnnnnn
Cut with Fsp I
nnnnnnTGC GCAnnnnnnn
nnnnnnACG CGTnnnnnnn

FIG. 2

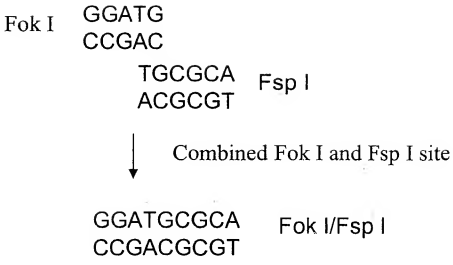

Fok I
GGATG
CCGAC
TGCGCA
ACGCGT
Fsp I
Combined Fok I and Fsp I site
GGATGCGCA
CCGACGCGT
Fok I/Fsp I

FIG. 3

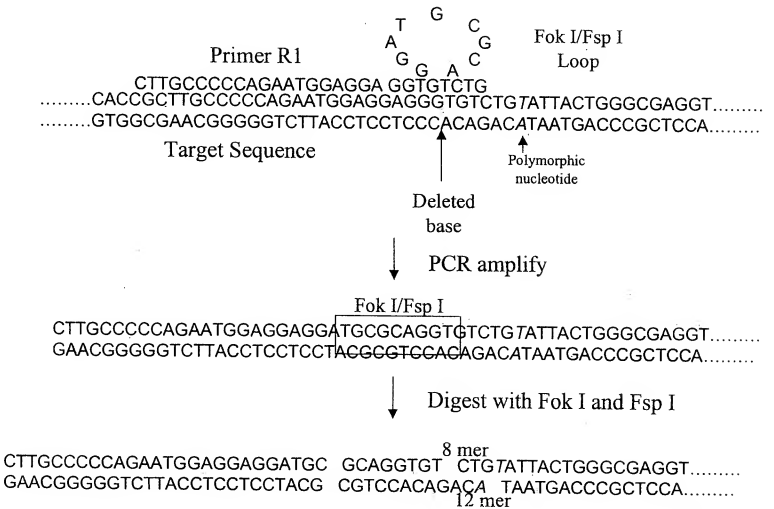

T
G
C
A
G
Fok I/Fsp I
Primer R1
G
C
Loop
G
A
CTTGCCCCCAGAATGGAGGA GGTGTCTG
.....CACCGCTTGCCCCCAGAATGGAGGAGGGTGTCTGTATTACTGGGCGAGGT.....
.....GTGGCGAACGGGGGTCTTACCTCCTCCCACAGACATAATGACCCGCTCCA.....
Target Sequence
Polymorphic nucleotide
Deleted base
PCR amplify
Fok I/Fsp I
CTTGCCCCCAGAATGGAGGAGGATGCGCAGGTGTCTGTATTACTGGGCGAGGT.....
GAACGGGGGTCTTACCTCCTCCTACGCGTCCACAGACATAATGACCCGCTCCA.....
Digest with Fok I and Fsp I
8 mer
CTTGCCCCCAGAATGGAGGAGGATGC GCAGGTGT CTGTATTACTGGGCGAGGT.....
GAACGGGGGTCTTACCTCCTCCTACG CGTCCACAGACA TAATGACCCGCTCCA.....
12 mer

FIG. 4

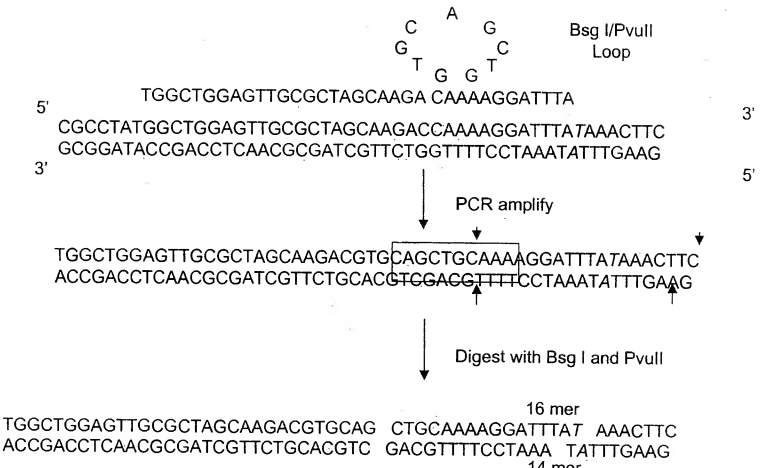

A
C
G
G
C
T
T
G G
Bsg I/PvuII
Loop
5'
TGGCTGGAGTTGCGCTAGCAAGA CAAAAGGATTTA
3'
CGCCTATGGCTGGAGTTGCGCTAGCAAGACCAAAAGGATTTATAAACTTC
GCGGATACCGACCTCAACGCGATCGTTCTGGTTTTCCTAAATATTTGAAG
3'
5'
PCR amplify
TGGCTGGAGTTGCGCTAGCAAGACGTGCAGCTGCAAAAGGATTTATAAACTTC
ACCGACCTCAACGCGATCGTTCTGCACGTCGACGTTTTCCTAAATATTTGAAG
Digest with Bsg I and PvuII
16 mer
TGGCTGGAGTTGCGCTAGCAAGACGTGCAG CTGCAAAAGGATTTAT AAACTTC
ACCGACCTCAACGCGATCGTTCTGCACGTC GACGTTTTCCTAAA TATTTGAAG
14 mer

FIG. 5

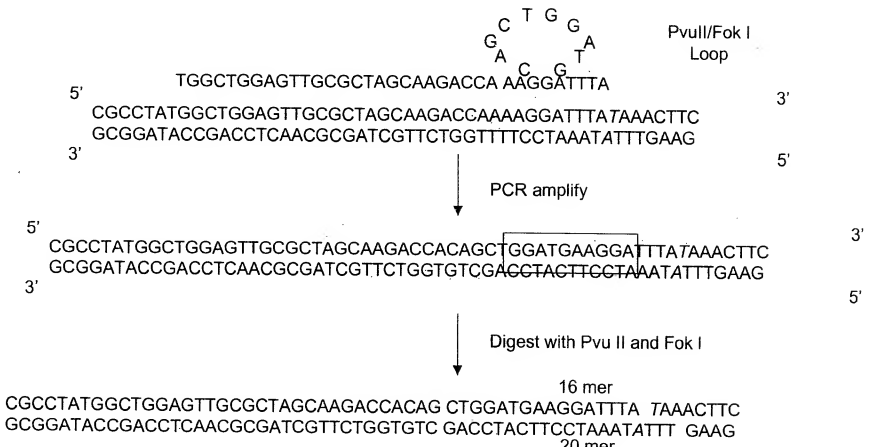

C T G G
G A C G A T
PvuII/Fok I
Loop
5'
TGGCTGGAGTTGCGCTAGCAAGACCA AAGGATTTA
3'
CGCCTATGGCTGGAGTTGCGCTAGCAAGACCAAAAGGATTTATAAACTTC
GCGGATACCGACCTCAACGCGATCGTTCTGGTTTTCCTAAATATTTGAAG
3'
5'
PCR amplify
5'
3'
CGCCTATGGCTGGAGTTGCGCTAGCAAGACCACAGCTGGATGAAGGATTTATAAACTTC
GCGGATACCGACCTCAACGCGATCGTTCTGGTGTCGACCTACTTCCTAAATATTTGAAG
3'
5'
Digest with Pvu II and Fok I
16 mer
CGCCTATGGCTGGAGTTGCGCTAGCAAGACCACAG CTGGATGAAGGATTTA TAAACTTC
GCGGATACCGACCTCAACGCGATCGTTCTGGTGTC GACCTACTTCCTAAATATTT GAAG
20 mer

FIG. 6

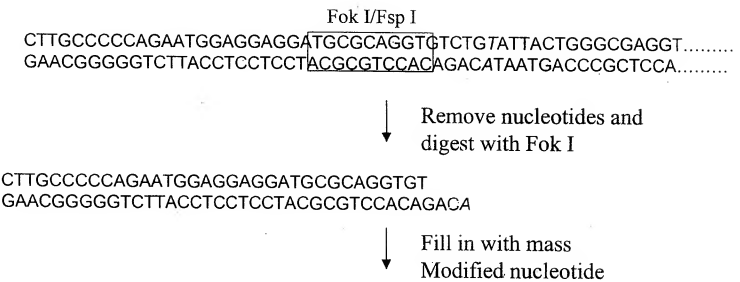

Fok I/Fsp I
CTTGCCCCCAGAATGGAGGAGGATGCGCAGGTGTCTGTATTACTGGGCGAGGT.....
GAACGGGGGTCTTACCTCCTCCTACGCGTCCACAGACATAATGACCCGCTCCA.....
Remove nucleotides and
digest with Fok I
CTTGCCCCCAGAATGGAGGAGGATGCGCAGGTGT
GAACGGGGGTCTTACCTCCTCCTACGCGTCCACAGACA
Fill in with mass
Modified nucleotide
CTTGCCCCCAGAATGGAGGAGGATGCGCAGGTGTCTGTmod
GAACGGGGGTCTTACCTCCTCCTACGCGTCCACAGACA

FIG 7


Bcg I
nnnnnnnnnnnnnnnnnCGAnnnnnnTGCnnnnnnnnnnnnnnnnn
nnnnnnnnnnnnnnnnnGCTnnnnnnACGnnnnnnnnnnnnnnnnn
Cleave with Bcg I
nnnnnnnnnnCGAnnnnnnTGCnnnnnnnnnnnn
nnnnnnnnnnnnGCTnnnnnnACGnnnnnnnnnn

FIG. 8

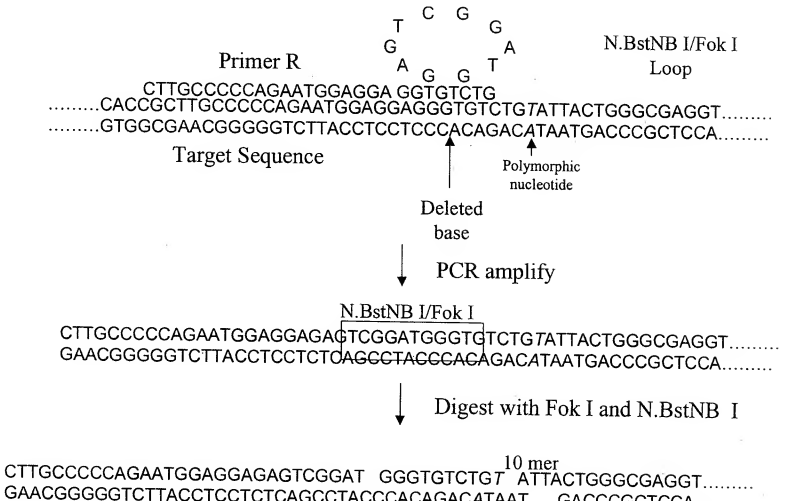

C G
T G
G A
A G G T
Primer R
N.BstNB I/Fok I
Loop
CTTGCCCCCAGAATGGAGGA GGTGTCTG
.....CACCGCTTGCCCCCAGAATGGAGGAGGGTGTCTGTATTACTGGGCGAGGT.....
.....GTGGCGAACGGGGGTCTTACCTCCTCCCACAGACATAATGACCCGCTCCA.....
Target Sequence
Polymorphic nucleotide
Deleted base
PCR amplify
N.BstNB I/Fok I
CTTGCCCCCAGAATGGAGGAGACTCGGATGGGTGTCTGTATTACTGGGCGAGGT.....
GAACGGGGGTCTTACCTCCTCTCAGCCTACCCACAGACATAATGACCCGCTCCA.....
Digest with Fok I and N.BstNB I
10 mer
CTTGCCCCCAGAATGGAGGAGAGTCGGAT GGGTGTCTGT ATTACTGGGCGAGGT.....
GAACGGGGGTCTTACCTCCTCTCAGCCTACCCACAGACATAAT GACCCGCTCCA.....

FIG. 9

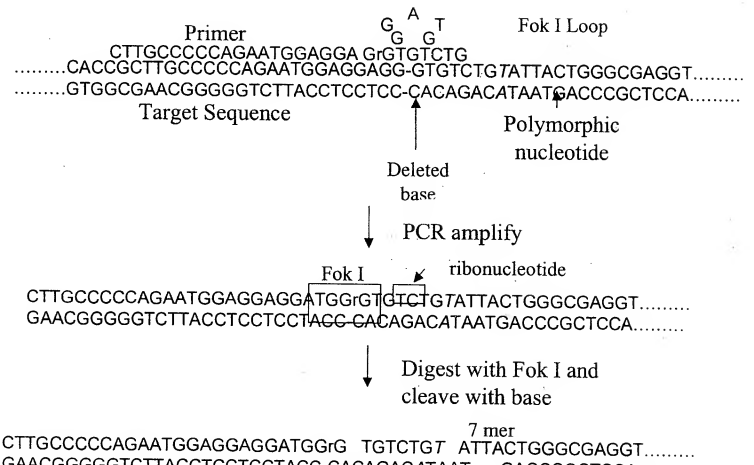

Primer
G A T
G G
Fok I Loop
CTTGCCCCCAGAATGGAGGA GrGTGTCTG
.....CACCGCTTGCCCCCAGAATGGAGGAGG-GTGTCTGTATTACTGGGCGAGGT.....
.....GTGGCGAACGGGGGTCTTACCTCCTCC-CACAGACATAATGACCCGCTCCA.....
Target Sequence
Polymorphic nucleotide
Deleted base
PCR amplify
Fok I
ribonucleotide
CTTGCCCCCAGAATGGAGGAGGATGGrGTGTCTGTATTACTGGGCGAGGT.....
GAACGGGGGTCTTACCTCCTCCTACC-CACAGACATAATGACCCGCTCCA.....
Digest with Fok I and cleave with base
7 mer
CTTGCCCCCAGAATGGAGGAGGATGGrG TGTCTGT ATTACTGGGCGAGGT.....
GAACGGGGGTCTTACCTCCTCCTACC-CACAGACATAAT GACCCGCTCCA.....

FIG. 10

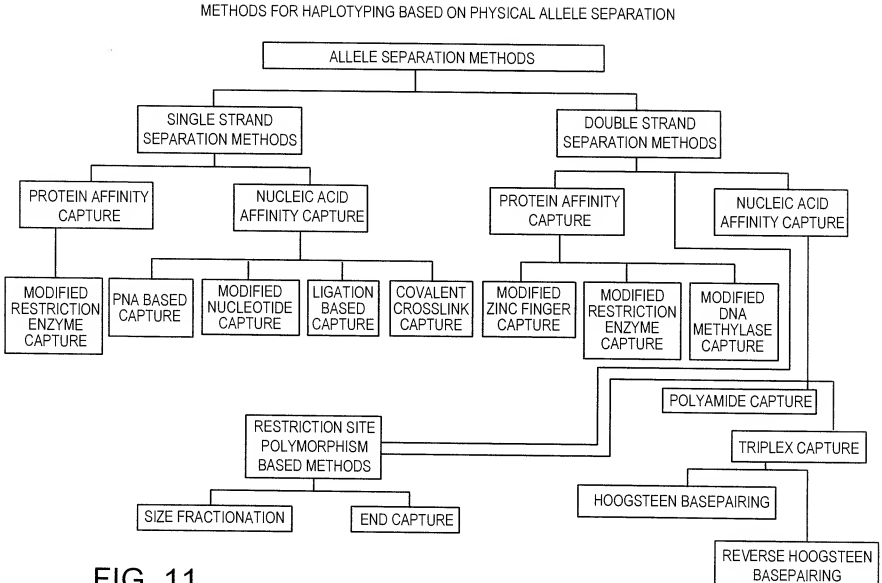

METHODS FOR HAPLOTYPING BASED ON PHYSICAL ALLELE SEPARATION
ALLELE SEPARATION METHODS
SINGLE STRAND SEPARATION METHODS
DOUBLE STRAND SEPARATION METHODS
PROTEIN AFFINITY CAPTURE
NUCLEIC ACID AFFINITY CAPTURE
PROTEIN AFFINITY CAPTURE
NUCLEIC ACID AFFINITY CAPTURE
MODIFIED RESTRICTION ENZYME CAPTURE
PNA BASED CAPTURE
MODIFIED NUCLEOTIDE CAPTURE
LIGATION BASED CAPTURE
COVALENT CROSSLINK CAPTURE
MODIFIED ZINC FINGER CAPTURE
MODIFIED RESTRICTION ENZYME CAPTURE
MODIFIED DNA METHYLASE CAPTURE
POLYAMIDE CAPTURE
RESTRICTION SITE POLYMORPHISM BASED METHODS
TRIPLEX CAPTURE
HOOGSTEEN BASEPAIRING
SIZE FRACTIONATION
END CAPTURE
REVERSE HOOGSTEEN BASEPAIRING

FIG. 11

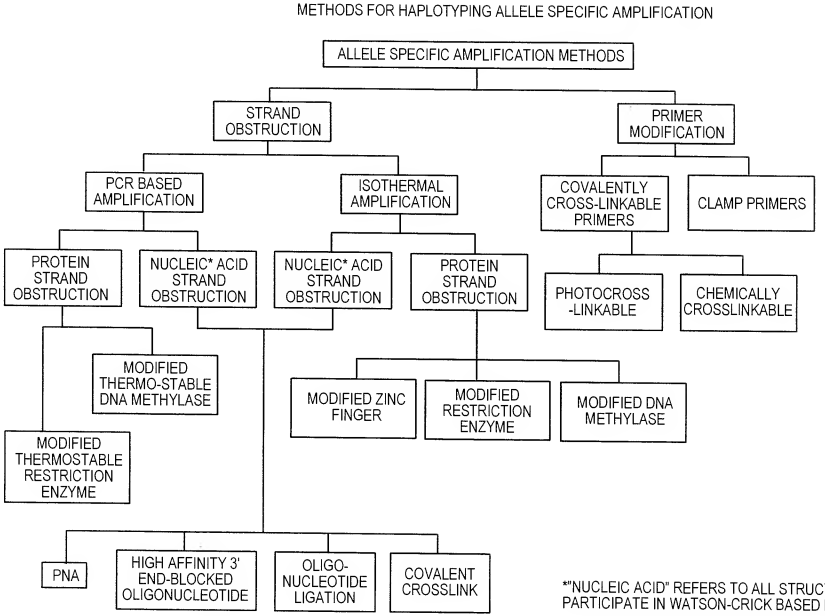

METHODS FOR HAPLOTYPING ALLELE SPECIFIC AMPLIFICATION
ALLELE SPECIFIC AMPLIFICATION METHODS
STRAND OBSTRUCTION
PRIMER MODIFICATION
PCR BASED AMPLIFICATION
ISOTHERMAL AMPLIFICATION
COVALENTLY CROSS-LINKABLE PRIMERS
CLAMP PRIMERS
PROTEIN STRAND OBSTRUCTION
NUCLEIC* ACID STRAND OBSTRUCTION
NUCLEIC* ACID STRAND OBSTRUCTION
PROTEIN STRAND OBSTRUCTION
PHOTOCROSS -LINKABLE
CHEMICALLY CROSSLINKABLE
MODIFIED THERMO-STABLE DNA METHYLASE
MODIFIED ZINC FINGER
MODIFIED RESTRICTION ENZYME
MODIFIED DNA METHYLASE
MODIFIED THERMOSTABLE RESTRICTION ENZYME
PNA
HIGH AFFINITY 3' END-BLOCKED OLIGONUCLEOTIDE
OLIGO-NUCLEOTIDE LIGATION
COVALENT CROSSLINK
*"NUCLEIC ACID" REFERS TO ALL STRUCTURES THAT PARTICIPATE IN WATSON-CRICK BASED PAIRING

FIG. 12

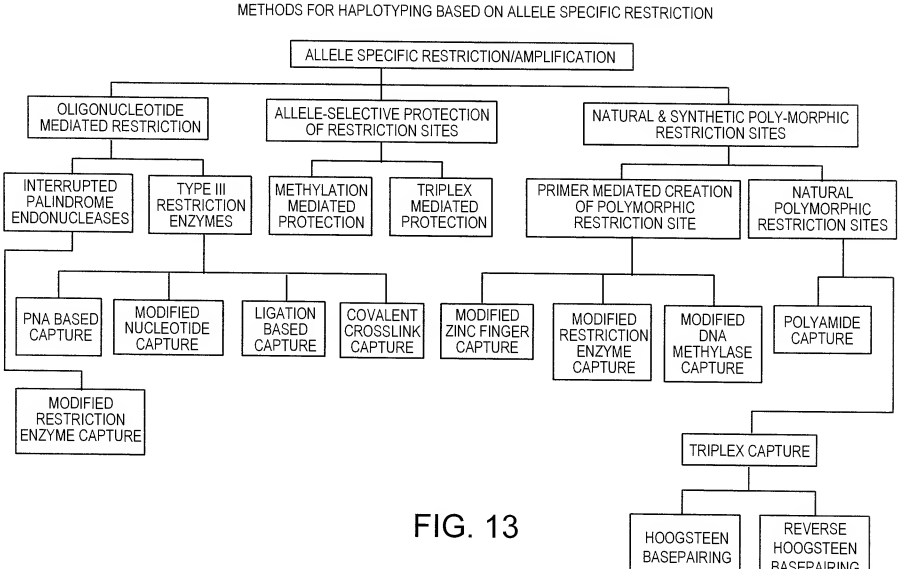

METHODS FOR HAPLOTYPING BASED ON ALLELE SPECIFIC RESTRICTION
ALLELE SPECIFIC RESTRICTION/AMPLIFICATION
OLIGONUCLEOTIDE MEDIATED RESTRICTION
ALLELE-SELECTIVE PROTECTION OF RESTRICTION SITES
NATURAL & SYNTHETIC POLY-MORPHIC RESTRICTION SITES
INTERRUPTED PALINDROME ENDONUCLEASES
TYPE III RESTRICTION ENZYMES
METHYLATION MEDIATED PROTECTION
TRIPLEX MEDIATED PROTECTION
PRIMER MEDIATED CREATION OF POLYMORPHIC RESTRICTION SITE
NATURAL POLYMORPHIC RESTRICTION SITES
PNA BASED CAPTURE
MODIFIED NUCLEOTIDE CAPTURE
LIGATION BASED CAPTURE
COVALENT CROSSLINK CAPTURE
MODIFIED ZINC FINGER CAPTURE
MODIFIED RESTRICTION ENZYME CAPTURE
MODIFIED DNA METHYLASE CAPTURE
POLYAMIDE CAPTURE
MODIFIED RESTRICTION ENZYME CAPTURE
TRIPLEX CAPTURE
HOOGSTEEN BASEPAIRING
REVERSE HOOGSTEEN BASEPAIRING

FIG. 13

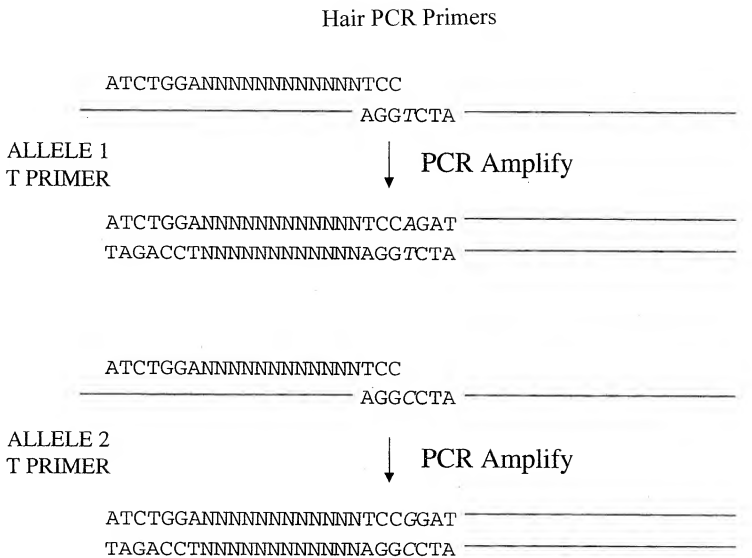

Hair PCR Primers
ATCTGGANNNNNNNNNNNNTCC
AGGTCTA
ALLELE 1
T PRIMER
PCR Amplify
ATCTGGANNNNNNNNNNNNNTCCAGAT
TAGACCTNNNNNNNNNNNNNAGGTCTA
ATCTGGANNNNNNNNNNNNTCC
AGGCCTA
ALLELE 2
T PRIMER
PCR Amplify
ATCTGGANNNNNNNNNNNNNTCCGGAT
TAGACCTNNNNNNNNNNNNNAGGCCTA

FIG. 14

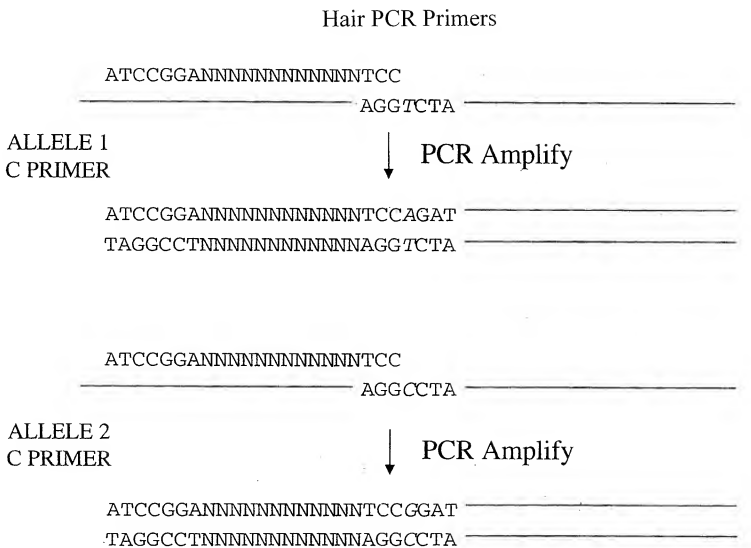

Hair PCR Primers
ATCCGGANNNNNNNNNNNNTCC
AGGTCTA
ALLELE 1
C PRIMER
PCR Amplify
ATCCGGANNNNNNNNNNNNTCCAGAT
TAGGCCTNNNNNNNNNNNNAGGTCTA
ATCCGGANNNNNNNNNNNNTCC
AGGCCTA
ALLELE 2
C PRIMER
PCR Amplify
ATCCGGANNNNNNNNNNNNTCCGGAT
TAGGCCTNNNNNNNNNNNNAGGCCTA

FIG. 15

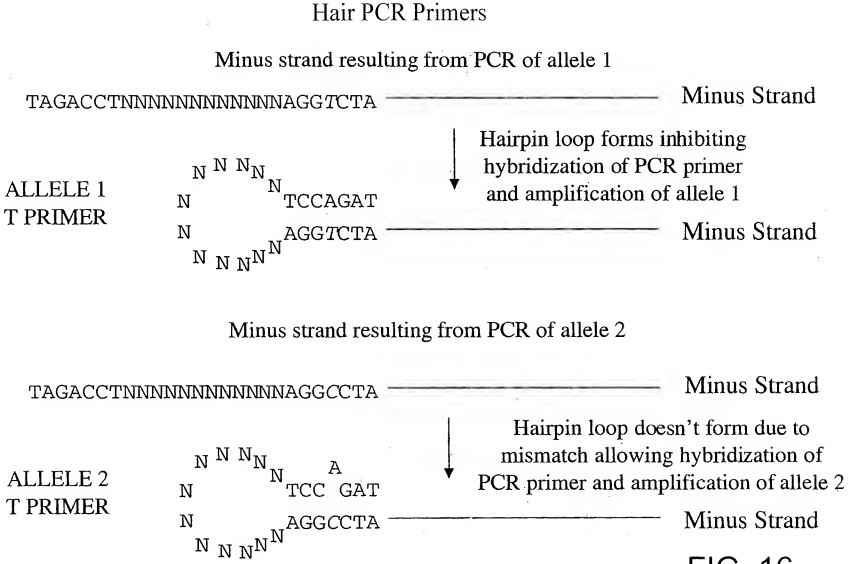

Hair PCR Primers
Minus strand resulting from PCR of allele 1
TAGACCTNNNNNNNNNNNNAGGTCTA
Minus Strand
Hairpin loop forms inhibiting hybridization of PCR primer and amplification of allele 1
ALLELE 1
T PRIMER
N N N N N
N TCCAGAT
N AGGTCTA
N N N N N
Minus Strand
Minus strand resulting from PCR of allele 2
TAGACCTNNNNNNNNNNNNAGGCCTA
Minus Strand
Hairpin loop doesn't form due to mismatch allowing hybridization of PCR primer and amplification of allele 2
ALLELE 2
T PRIMER
N N N N N A
N TCC GAT
N AGGCCTA
N N N N N
Minus Strand

FIG. 16

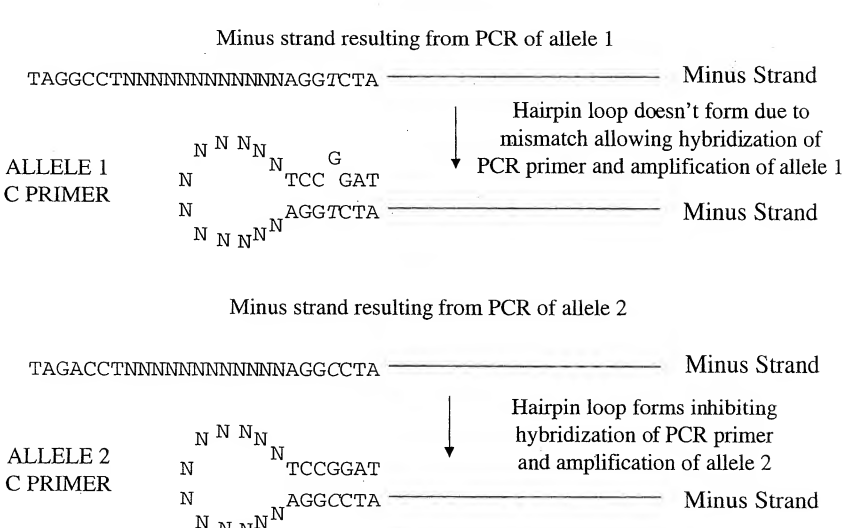

Hair PCR Primers
Minus strand resulting from PCR of allele 1
TAGGCCTNNNNNNNNNNNNAGGTCTA
Minus Strand
Hairpin loop doesn't form due to mismatch allowing hybridization of PCR primer and amplification of allele 1
ALLELE 1 C PRIMER
N N N N N G
N TCC GAT
N AGGTCTA
Minus Strand
N N N N N
Minus strand resulting from PCR of allele 2
TAGACCTNNNNNNNNNNNNAGGCCTA
Minus Strand
Hairpin loop forms inhibiting hybridization of PCR primer and amplification of allele 2
ALLELE 2 C PRIMER
N N N N N
N TCCGGAT
N AGGCCTA
Minus Strand
N N N N N

FIG. 17

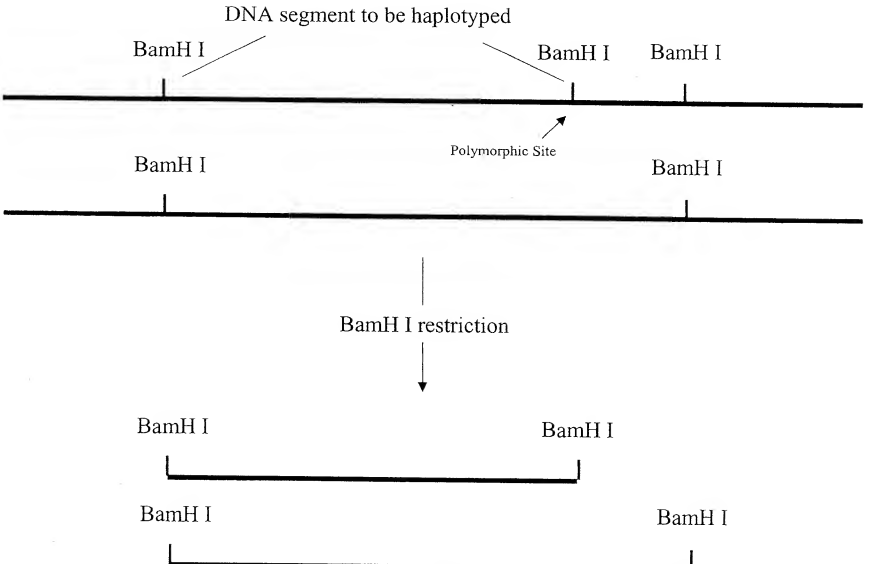

DNA segment to be haplotyped
BamH I
BamH I
BamH I
Polymorphic Site
BamH I
BamH I
BamH I restriction
BamH I
BamH I
BamH I
BamH I

FIG 18

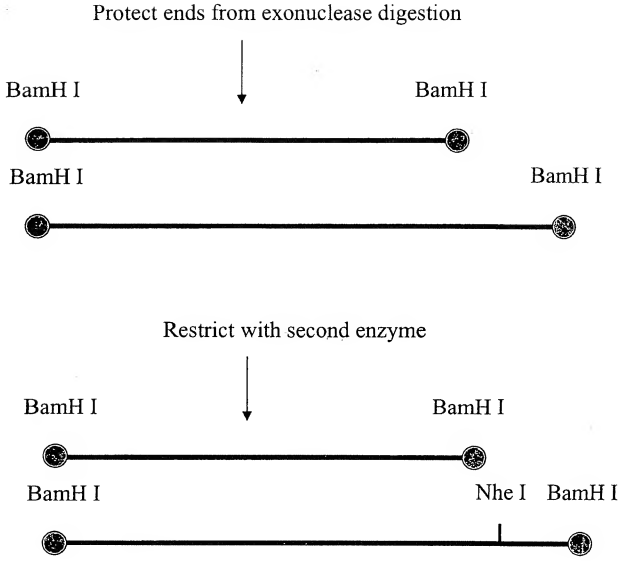

Protect ends from exonuclease digestion
BamH I
BamH I
BamH I
BamH I
Restrict with second enzyme
BamH I
BamH I
BamH I
Nhe I
BamH I

FIG. 19

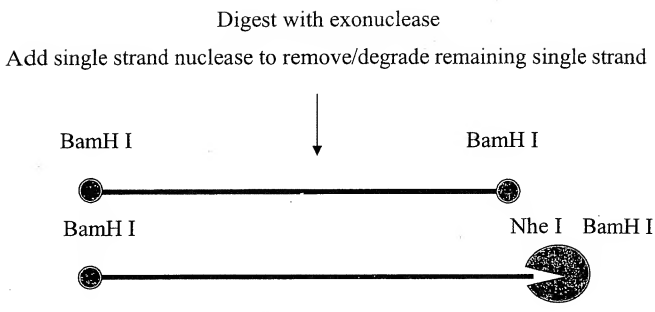

Digest with exonuclease
Add single strand nuclease to remove/degrade remaining single strand
BamH I
BamH I
BamH I
Nhe I
BamH I

FIG. 20

Dihydropyrimidine dehydrogenase (DPD) polymorphisms used in haplotyping assay.

DPD polymorphisms

Base	Nucleotide	Amino Acid
186	T	Cys
	C	Arg
597	A	Met
	G	Val

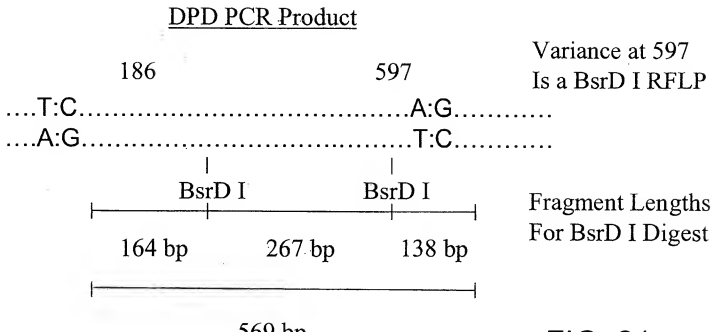


FIG. 21

Allele Specific Primers for DPD

A.

DPD Primers	DPDASCF	5' acacagactcatgcaactctg 3'
	DPDASTF	5' acgcagactcatgcaactctg 3'
	DPDNSF	5' actcatgcaactctg 3'

B.

DPD Sequence

5' actcatgcaactctg[T or C]gttccacttcggccaagaa 3'

3' tgagtacgttgagac[A or G]caaggtgaagccggttctt 5'

FIG. 22

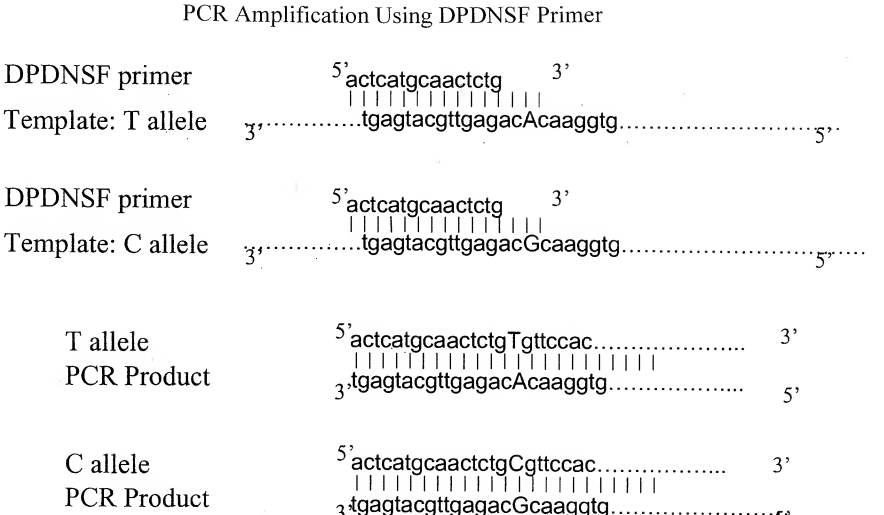

PCR Amplification Using DPDNSF Primer
DPDNSF primer
5'actcatgcaactctg 3'
Template: T allele
3'.....tgagtacgttgagacAcaaggtg.....5'
DPDNSF primer
5'actcatgcaactctg 3'
Template: C allele
3'.....tgagtacgttgagacGcaaggtg.....5'
T allele
PCR Product
5'actcatgcaactctgTgttccac..... 3'
3'tgagtacgttgagacAcaaggtg..... 5'
C allele
PCR Product
5'actcatgcaactctgCgttccac..... 3'
3'tgagtacgttgagacGcaaggtg.....5'

FIG. 23

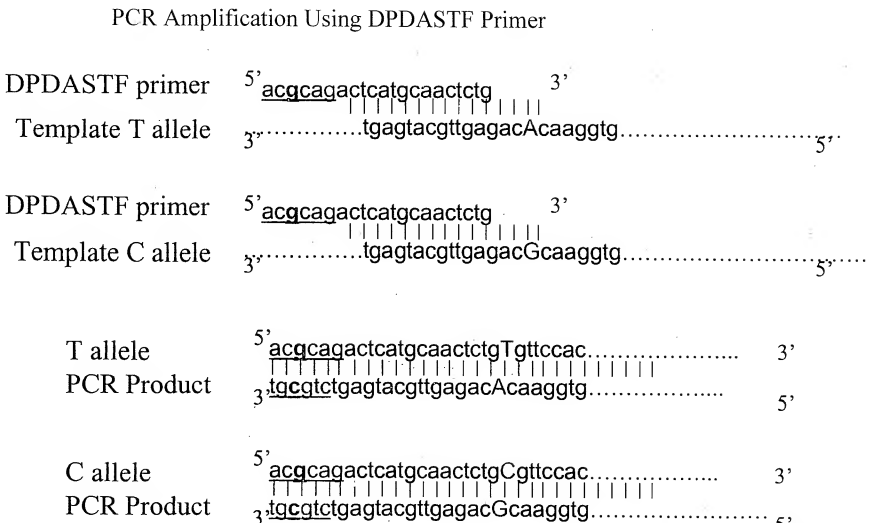

PCR Amplification Using DPDASTF Primer
DPDASTF primer
5' acgcagactcatgcaactctg 3'
Template T allele
3' tgagtacgttgagacAcaaggtg 5'
DPDASTF primer
5' acgcagactcatgcaactctg 3'
Template C allele
3' tgagtacgttgagacGcaaggtg 5'
T allele
PCR Product
5' acgcagactcatgcaactctgTgttccac 3'
3' tgcgtctgagtacgttgagacAcaaggtg 5'
C allele
PCR Product
5' acgcagactcatgcaactctgCgttccac 3'
3' tgcgtctgagtacgttgagacGcaaggtg 5'

FIG. 24

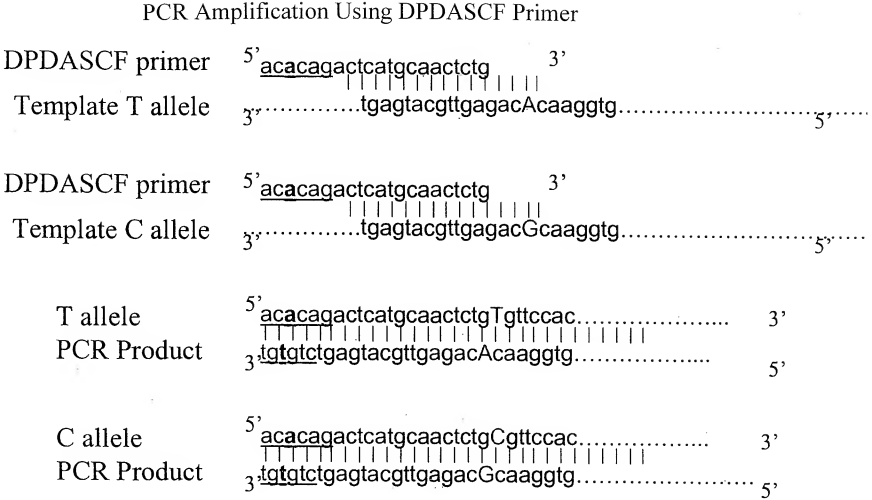
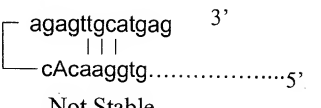

PCR Amplification Using DPDASCF Primer
DPDASCF primer
5' acacagactcatgcaactctg 3'
Template T allele
3' .....tgagtacgttgagacAcaaggtg..... 5'
DPDASCF primer
5' acacagactcatgcaactctg 3'
Template C allele
3' .....tgagtacgttgagacGcaaggtg..... 5'
T allele
PCR Product
5' acacagactcatgcaactctgTgttccac..... 3'
3' tgtgtctgagtacgttgagacAcaaggtg..... 5'
C allele
PCR Product
5' acacagactcatgcaactctgCgttccac..... 3'
3' tgtgtctgagtacgttgagacGcaaggtg..... 5'

FIG. 25

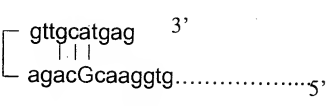
Hairpin Structures for PCR Products Generated Using DPDNSF Primer

Hairpin Structure T
Allele Reverse Strand



Not Stable

Hairpin Structure C
Allele Reverse Strand



Tm = 40°C

FIG. 26

Hairpin Structures for PCR Products Generated Using υPDASCF Primer

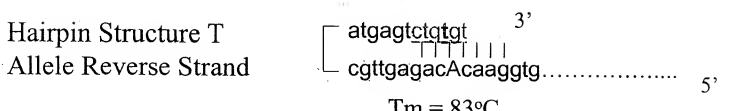


FIG. 27

Hairpin Structures for PCR Products Generated Using DPDASTF Primer

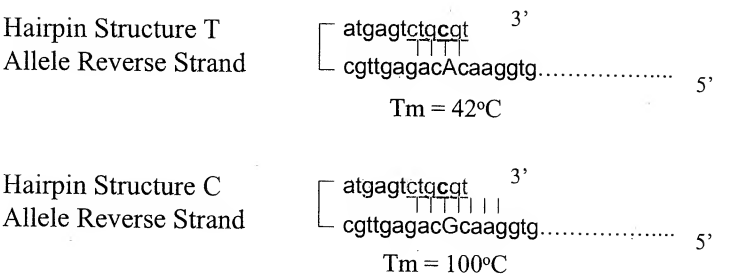


FIG. 28

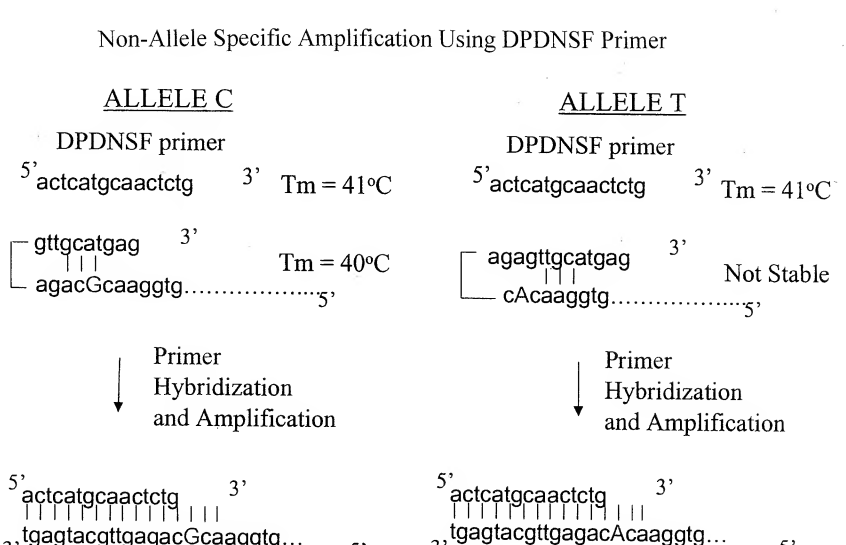

Non-Allele Specific Amplification Using DPDNSF Primer
ALLELE C
DPDNSF primer
5' actcatgcaactctg 3'
Tm = 41°C
gttgcatgag 3'
agacGcaaggtg.....5'
Tm = 40°C
Primer Hybridization and Amplification
5' actcatgcaactctg 3'
3' tgagtacgttgagacGcaaggtg... 5'
ALLELE T
DPDNSF primer
5' actcatgcaactctg 3'
Tm = 41°C
agagttgcatgag 3'
cAcaaggtg.....5'
Not Stable
Primer Hybridization and Amplification
5' actcatgcaactctg 3'
3' tgagtacgttgagacAcaaggtg... 5'

FIG. 29

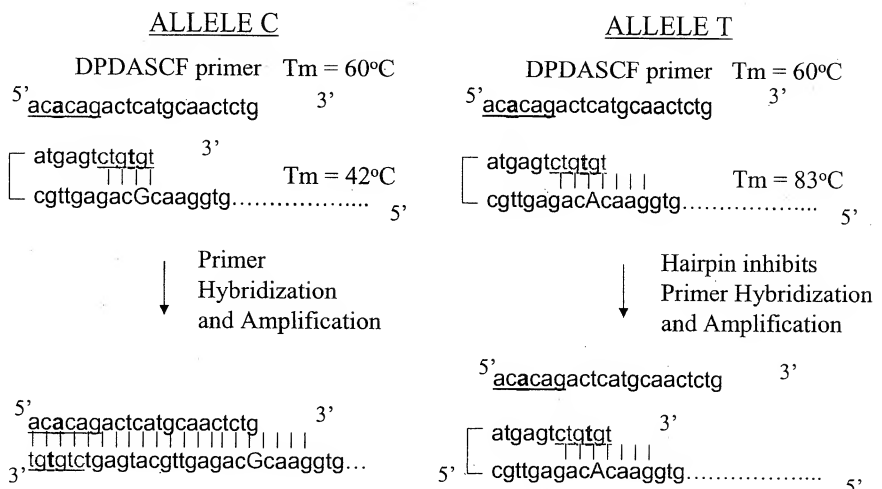

Allele Specific Amplification Using DPDASCF Primer
ALLELE C
DPDASCF primer Tm = 60°C
5' acacagactcatgcaactctg 3'
atgagtctgtgt 3'
cgttgagacGcaaggtg..... 5'
Tm = 42°C
Primer
Hybridization
and Amplification
5' acacagactcatgcaactctg 3'
3' tgtgtctgagtacgttgagacGcaaggtg...
ALLELE T
DPDASCF primer Tm = 60°C
5' acacagactcatgcaactctg 3'
atgagtctgtgt
cgttgagacAcaaggtg..... 5'
Tm = 83°C
Hairpin inhibits
Primer Hybridization
and Amplification
5' acacagactcatgcaactctg 3'
atgagtctgtgt 3'
5' cgttgagacAcaaggtg..... 5'

FIG 30

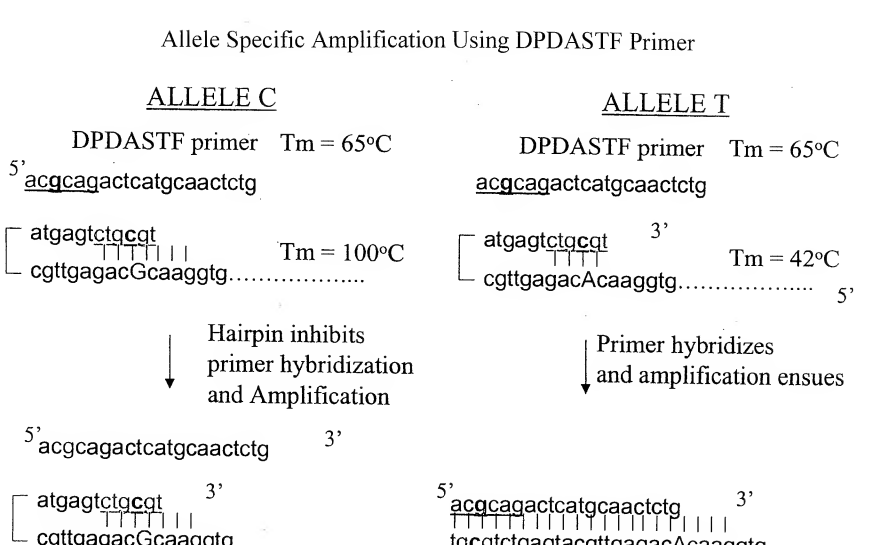

Allele Specific Amplification Using DPDASTF Primer
ALLELE C
DPDASTF primer Tm = 65°C
5' acgcagactcatgcaactctg
atgagtctgcgt
cgttgagacGcaaggtg.....
Tm = 100°C
Hairpin inhibits primer hybridization and Amplification
5' acgcagactcatgcaactctg 3'
atgagtctgcgt 3'
cgttgagacGcaaggtg..... 5'
ALLELE T
DPDASTF primer Tm = 65°C
acgcagactcatgcaactctg
atgagtctgcgt 3'
cgttgagacAcaaggtg..... 5'
Tm = 42°C
Primer hybridizes and amplification ensues
5' acgcagactcatgcaactctg 3'
3' tgcgtctgagtacgttgagacAcaaggtg... 5'

FIG. 31

Allele Specific Amplification of a Heterozygous Sample with Haplotype T^{186}, A^{597} and C^{186}, G^{597}

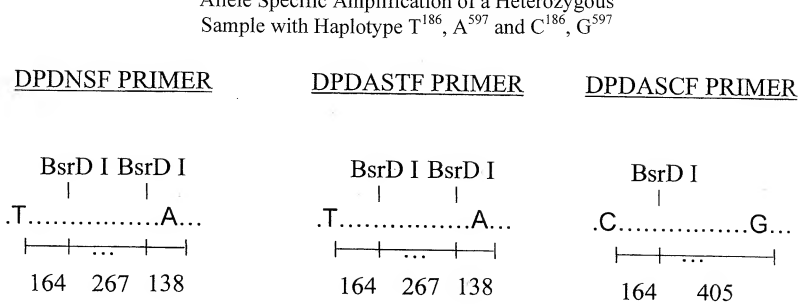


FIG. 32

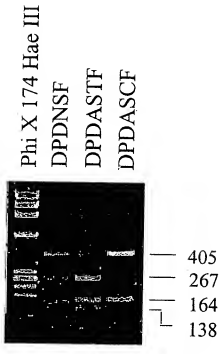


FIG. 33

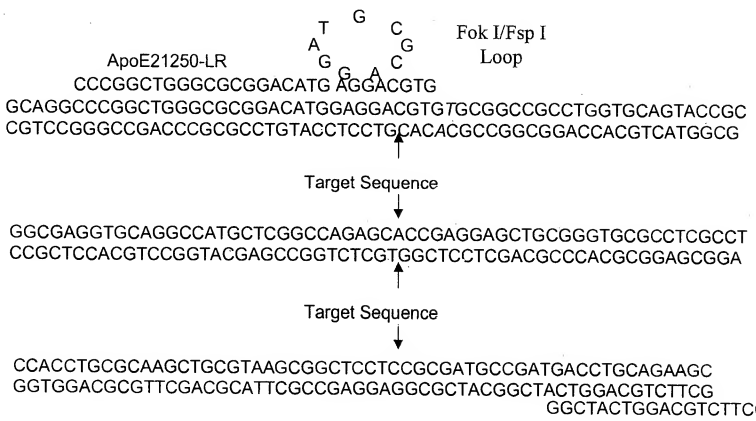

T
G
C
A
G
Fok I/Fsp I
Loop
ApoE21250-LR
G
C
G
A
CCCGGCTGGGCGCGGACATG AGGACGTG
GCAGGCCCGGCTGGGCGCGGACATGGAGGACGTGTGCGGCCGCCTGGTGCAGTACCGC
CGTCCGGGCCGACCCGCGCCTGTACCTCCTGCACACGCCGGCGGACCACGTCATGGCG
Target Sequence
GGCGAGGTGCAGGCCATGCTCGGCCAGAGCACCGAGGAGCTGCGGGTGCGCCTCGCCT
CCGCTCCACGTCCGGTACGAGCCGGTCTCGTGGCTCCTCGACGCCCACGCGGAGCGGA
Target Sequence
CCACCTGCGCAAGCTGCGTAAGCGGCTCCTCCGCGATGCCGATGACCTGCAGAAGC
GGTGGACGCGTTCGACGCATTCGCCGAGGAGGCGCTACGGCTACTGGACGTCTTCG
GGCTACTGGACGTCTTCG
ApoE21250-LR

FIG. 34

T Allele Amplicon

CCCGGCTGGGCGCGGACATGGGATGCGCAAGGACGTGTGCGGCCGCCTGGTGCAGTAC
GGGCCGACCCGCGCCTGTACCCTACGCGTTCCTGCACACGCCGCGGCGGACCACGTCATG

CGCGGCGAGGTGCAGGCCATGCTCGGCCAGAGCACCGAGGAGCTGCGGGTGCGCCTCG
GCGCCGCTCCACGTCCGGTACGAGCCGGTCTCGTGGCTCCTCGACGCCCACGCGGAGC

CCTCCACCTGCGCAAGCTGCGTAAGCGGCTCCTCCGCGATGCCGATGACCTGCAGAAGC
GGAGGTGGACGCGTTCGACGCATTCGCCGAGGAGGCGCTACGGCTACTGGACGTCTTCG

C Allele Amplicon

CCCGGCTGGGCGCGGACATGGGATGCGCAAGGACGTGCGCGGCCGCCTGGTGCAGTAC
GGGCCGACCCGCGCCTGTACCCTACGCGTTCCTGCACGCGCCGCGGCGGACCACGTCATG

CGCGGCGAGGTGCAGGCCATGCTCGGCCAGAGCACCGAGGAGCTGCGGGTGCGCCTCG
GCGCCGCTCCACGTCCGGTACGAGCCGGTCTCGTGGCTCCTCGACGCCCACGCGGAGC

CCTCCACCTGCGCAAGCTGCGTAAGCGGCTCCTCCGCGATGCCGATGACCTGCAGAAGC
GGAGGTGGACGCGTTCGACGCATTCGCCGAGGAGGCGCTACGGCTACTGGACGTCTTCG

FIG. 35

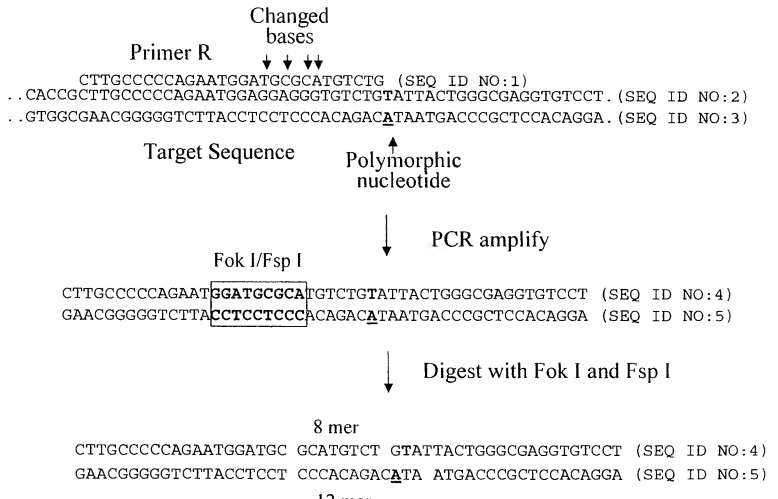

Changed bases
Primer R
CTTGCCCCCAGAATGGATGCGCATGTCTG (SEQ ID NO:1)
..CACCGCTTGCCCCCAGAATGGAGGAGGGTGTCTGTATTACTGGGCGAGGTGTCCT.(SEQ ID NO:2)
..GTGGCGAACGGGGGTCTTACCTCCTCCCACAGACATAATGACCCGCTCCACAGGA.(SEQ ID NO:3)
Target Sequence
Polymorphic nucleotide
PCR amplify
Fok I/Fsp I
CTTGCCCCCAGAATGGATGCGCATGTCTGTATTACTGGGCGAGGTGTCCT (SEQ ID NO:4)
GAACGGGGGTCTTACCTCCTCCCACAGACATAATGACCCGCTCCACAGGA (SEQ ID NO:5)
Digest with Fok I and Fsp I
8 mer
CTTGCCCCCAGAATGGATGC GCATGTCT GTATTACTGGGCGAGGTGTCCT (SEQ ID NO:4)
GAACGGGGGTCTTACCTCCT CCCACAGACATA ATGACCCGCTCCACAGGA (SEQ ID NO:5)
12 mer

FIG. 1

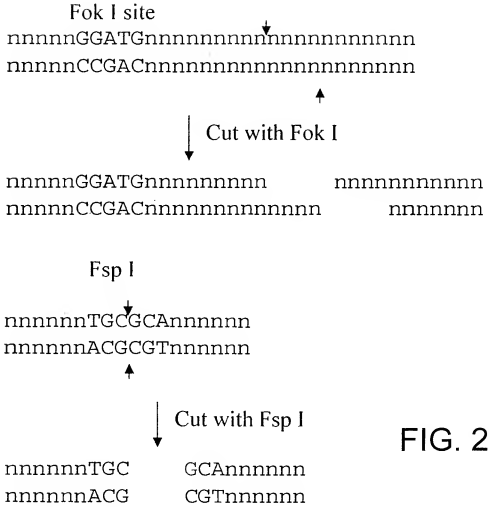

Fok I site
nnnnnGGATGnnnnnnnnnnnnnnnnnnnn
nnnnnCCGACnnnnnnnnnnnnnnnnnnnn
Cut with Fok I
nnnnnGGATGnnnnnnnnn
nnnnnnnnnnn
nnnnnCCGACnnnnnnnnnnnnnn
nnnnnnn
Fsp I
nnnnnnTGCGCAnnnnnn
nnnnnnACGCGTnnnnnn
Cut with Fsp I
nnnnnnTGC
GCAnnnnnn
nnnnnnACG
CGTnnnnnn

FIG. 2

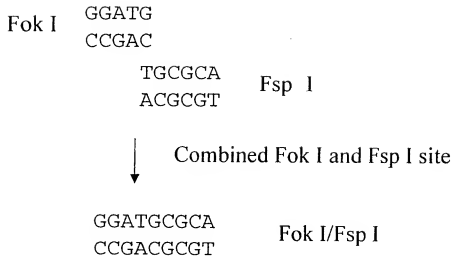

Fok I
GGATG
CCGAC
TGCGCA
ACGCGT
Fsp I
Combined Fok I and Fsp I site
GGATGCGCA
CCGACGCGT
Fok I/Fsp I

FIG. 3

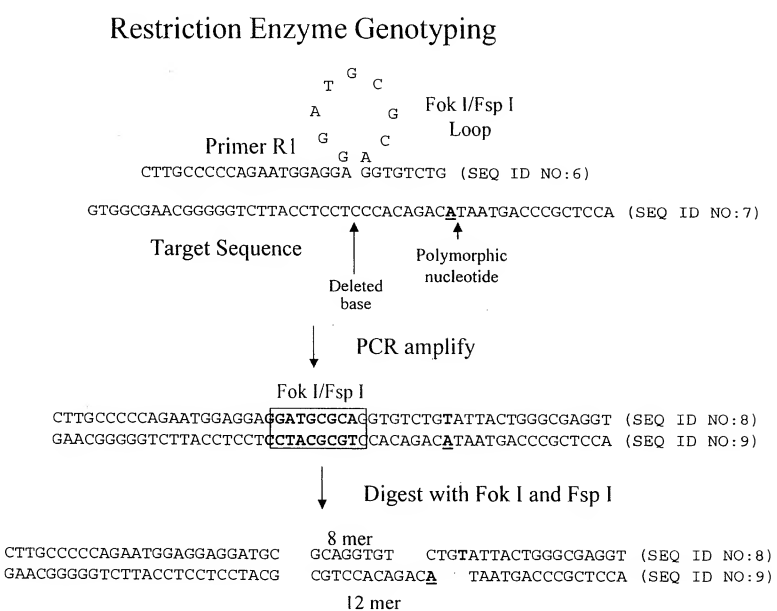

Restriction Enzyme Genotyping
T G C
A G
G C
G A
Fok I/Fsp I
Loop
Primer R1
CTTGCCCCCAGAATGGAGGA GGTGTCTG (SEQ ID NO:6)
GTGGCGAACGGGGGTCTTACCTCCTCCCACAGACATAATGACCCGCTCCA (SEQ ID NO:7)
Target Sequence
Polymorphic nucleotide
Deleted base
PCR amplify
Fok I/Fsp I
CTTGCCCCCAGAATGGAGGAGGATGCGCAGGTGTCTGTATTACTGGGCGAGGT (SEQ ID NO:8)
GAACGGGGGTCTTACCTCCTCCTACGCGTCCACAGACATAATGACCCGCTCCA (SEQ ID NO:9)
Digest with Fok I and Fsp I
8 mer
CTTGCCCCCAGAATGGAGGAGGATGC GCAGGTGT CTGTATTACTGGGCGAGGT (SEQ ID NO:8)
GAACGGGGGTCTTACCTCCTCCTACG CGTCCACAGACA TAATGACCCGCTCCA (SEQ ID NO:9)
12 mer

FIG. 4

Introduction of Bsg I and Pvu II sites during PCR by loop followed by endonuclease digestion.

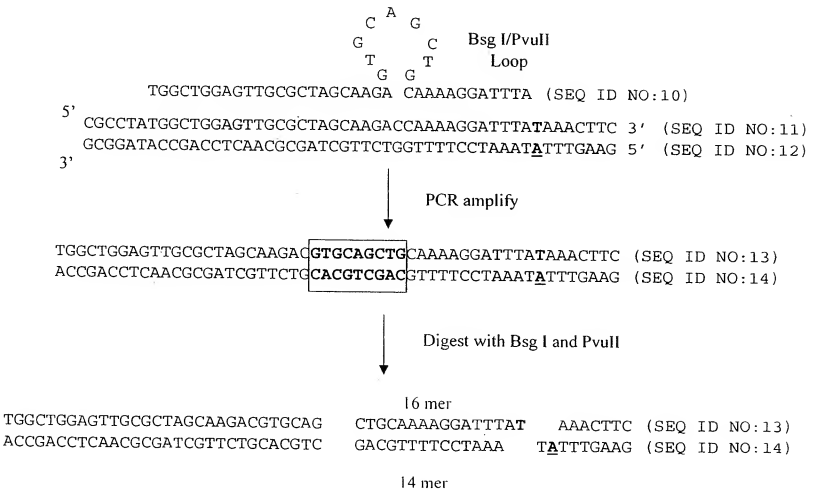


FIG. 5

Introduction of Fok I and Pvu II sites during PCR by loop followed by endonuclease digestion

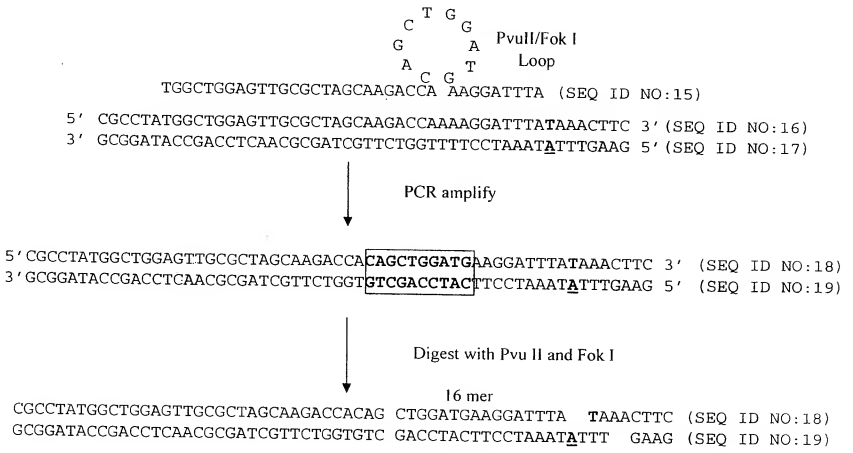


FIG. 6

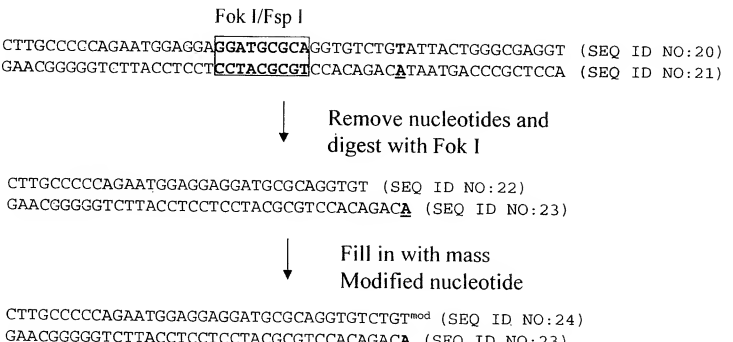

Fok I/Fsp I
CTTGCCCCCAGAATGGAGGAGGATGCGCAGGTGTCTGTATTACTGGGCGAGGT (SEQ ID NO:20)
GAACGGGGGTCTTACCTCCTCCTACGCGTCCACAGACATAATGACCCGCTCCA (SEQ ID NO:21)
Remove nucleotides and digest with Fok I
CTTGCCCCCAGAATGGAGGAGGATGCGCAGGTGT (SEQ ID NO:22)
GAACGGGGGTCTTACCTCCTCCTACGCGTCCACAGACA (SEQ ID NO:23)
Fill in with mass Modified nucleotide
CTTGCCCCCAGAATGGAGGAGGATGCGCAGGTGTCTGTmod (SEQ ID NO:24)
GAACGGGGGTCTTACCTCCTCCTACGCGTCCACAGACA (SEQ ID NO:23)

FIG. 7

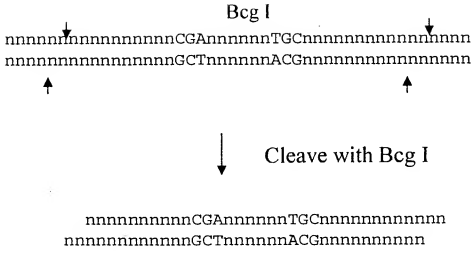

Bcg I
nnnnnnnnnnnnnnnnnCGAnnnnnnTGCnnnnnnnnnnnnnnnnn
nnnnnnnnnnnnnnnnnGCTnnnnnnACGnnnnnnnnnnnnnnnnn
Cleave with Bcg I
nnnnnnnnnnCGAnnnnnnTGCnnnnnnnnnnnn
nnnnnnnnnnnnGCTnnnnnnACGnnnnnnnnnn

FIG. 8

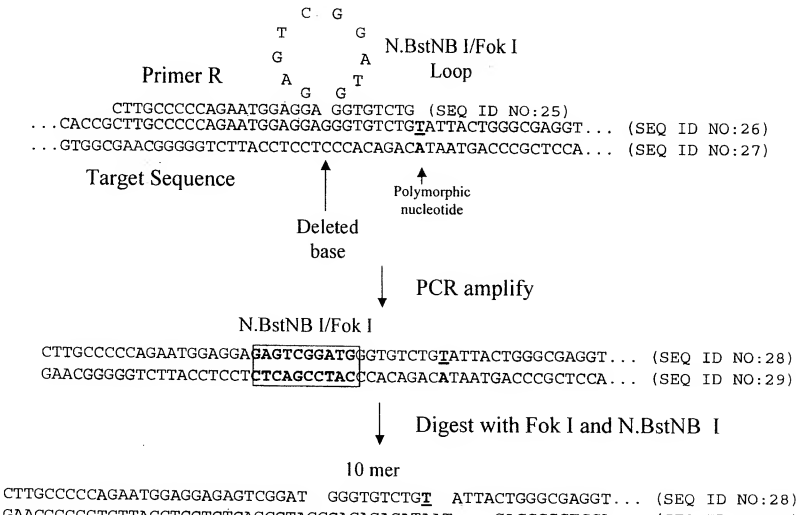

C G
T G
G A
A T
G G
N.BstNB I/Fok I
Loop
Primer R
CTTGCCCCCAGAATGGAGGA GGTGTCTG (SEQ ID NO:25)
...CACCGCTTGCCCCCAGAATGGAGGAGGGTGTCTGTATTACTGGGCGAGGT... (SEQ ID NO:26)
...GTGGCGAACGGGGGTCTTACCTCCTCCCACAGACATAATGACCCGCTCCA... (SEQ ID NO:27)
Target Sequence
Polymorphic nucleotide
Deleted base
PCR amplify
N.BstNB I/Fok I
CTTGCCCCCAGAATGGAGGAGAGTCGGATGGGTGTCTGTATTACTGGGCGAGGT... (SEQ ID NO:28)
GAACGGGGGTCTTACCTCCTCTCAGCCTACCCACAGACATAATGACCCGCTCCA... (SEQ ID NO:29)
Digest with Fok I and N.BstNB I
10 mer
CTTGCCCCCAGAATGGAGGAGAGTCGGAT GGGTGTCTGT ATTACTGGGCGAGGT... (SEQ ID NO:28)
GAACGGGGGTCTTACCTCCTCTCAGCCTACCCACAGACATAAT GACCCGCTCCA... (SEQ ID NO:29)

FIG. 9

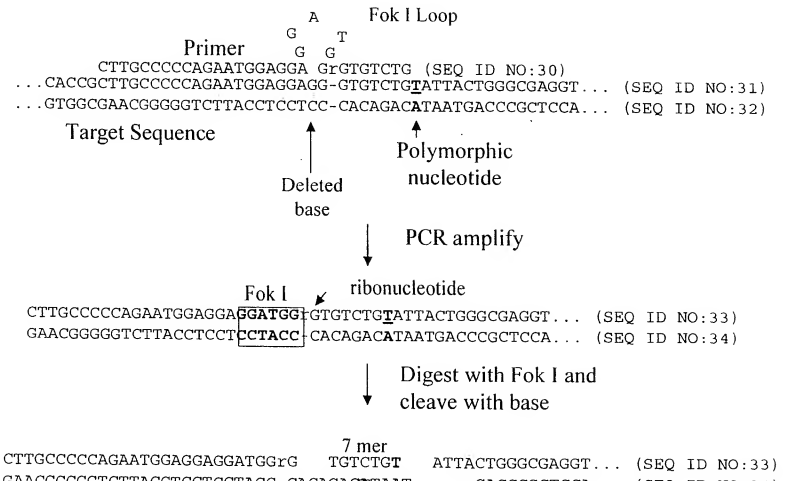

A
Fok I Loop
G
T
Primer
G G
CTTGCCCCCAGAATGGAGGA GrGTGTCTG (SEQ ID NO:30)
...CACCGCTTGCCCCCAGAATGGAGGAGG-GTGTCTGTATTACTGGGCGAGGT... (SEQ ID NO:31)
...GTGGCGAACGGGGGTCTTACCTCCTCC-CACAGACATAATGACCCGCTCCA... (SEQ ID NO:32)
Target Sequence
Deleted base
Polymorphic nucleotide
PCR amplify
Fok I
ribonucleotide
CTTGCCCCCAGAATGGAGGAGGATGGrGTGTCTGTATTACTGGGCGAGGT... (SEQ ID NO:33)
GAACGGGGGTCTTACCTCCTCCTACC-CACAGACATAATGACCCGCTCCA... (SEQ ID NO:34)
Digest with Fok I and cleave with base
7 mer
CTTGCCCCCAGAATGGAGGAGGATGGrG TGTCTGT ATTACTGGGCGAGGT... (SEQ ID NO:33)
GAACGGGGGTCTTACCTCCTCCTACC-CACAGACATAAT GACCCGCTCCA... (SEQ ID NO:34)

FIG. 10

METHODS FOR HAPLOTYPING BASED ON PHYSICAL ALLELE SEPARATION

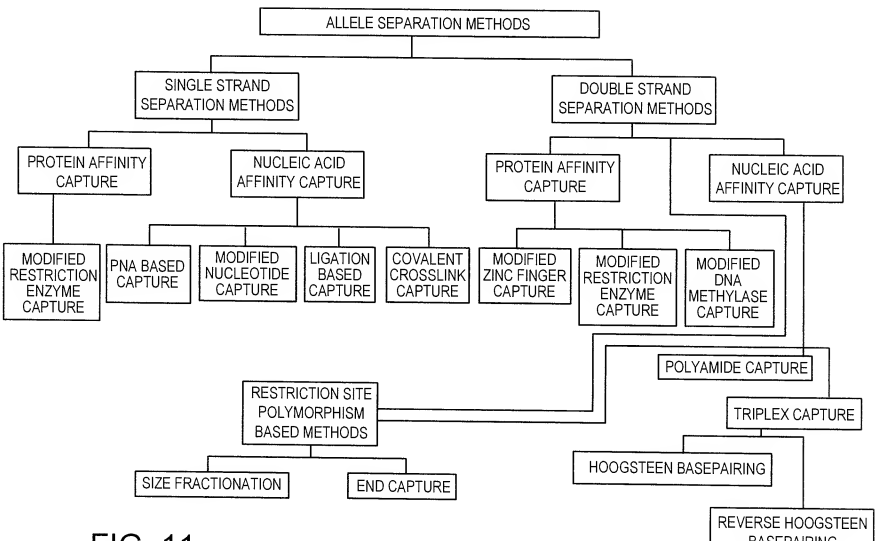
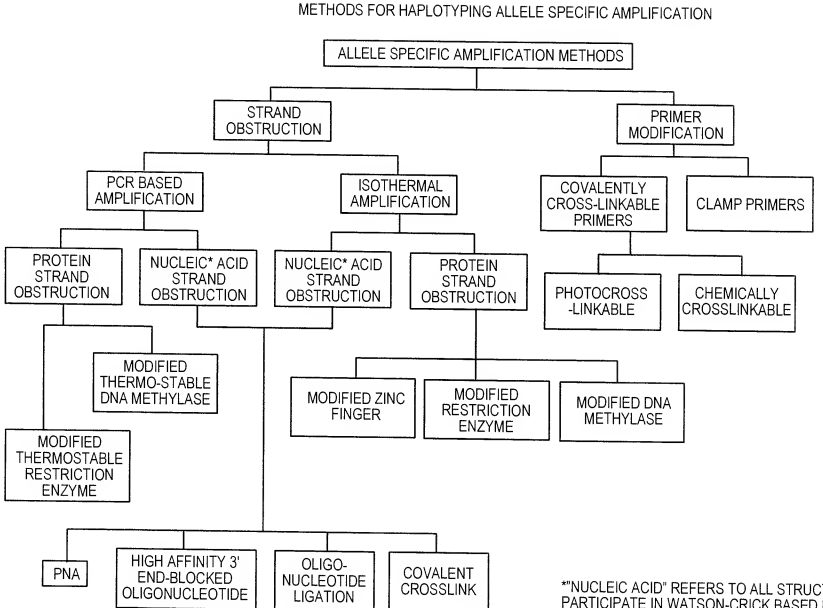


FIG. 11



*"NUCLEIC ACID" REFERS TO ALL STRUCTURES THAT PARTICIPATE IN WATSON-CRICK BASED PAIRING

FIG. 12

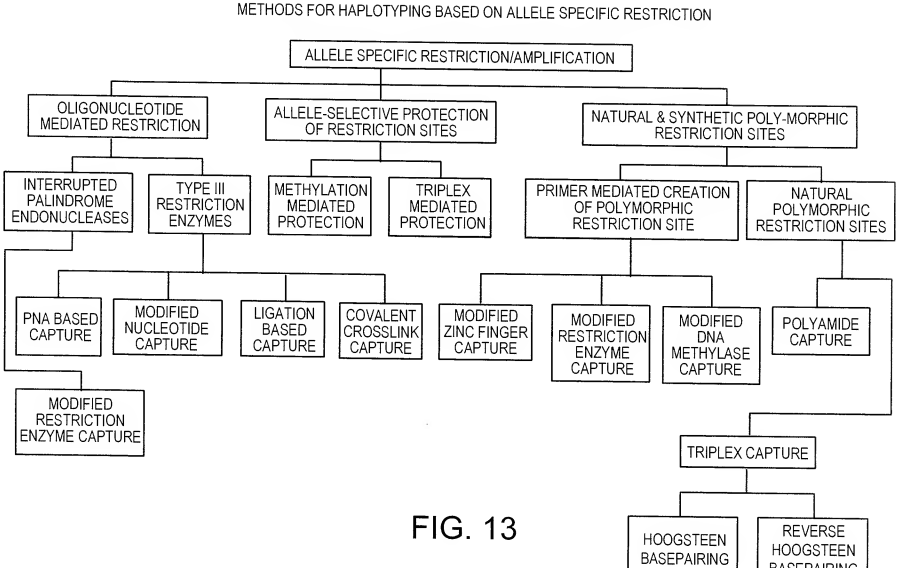

METHODS FOR HAPLOTYPING BASED ON ALLELE SPECIFIC RESTRICTION
ALLELE SPECIFIC RESTRICTION/AMPLIFICATION
OLIGONUCLEOTIDE MEDIATED RESTRICTION
ALLELE-SELECTIVE PROTECTION OF RESTRICTION SITES
NATURAL & SYNTHETIC POLY-MORPHIC RESTRICTION SITES
INTERRUPTED PALINDROME ENDONUCLEASES
TYPE III RESTRICTION ENZYMES
METHYLATION MEDIATED PROTECTION
TRIPLEX MEDIATED PROTECTION
PRIMER MEDIATED CREATION OF POLYMORPHIC RESTRICTION SITE
NATURAL POLYMORPHIC RESTRICTION SITES
PNA BASED CAPTURE
MODIFIED NUCLEOTIDE CAPTURE
LIGATION BASED CAPTURE
COVALENT CROSSLINK CAPTURE
MODIFIED ZINC FINGER CAPTURE
MODIFIED RESTRICTION ENZYME CAPTURE
MODIFIED DNA METHYLASE CAPTURE
POLYAMIDE CAPTURE
MODIFIED RESTRICTION ENZYME CAPTURE
TRIPLEX CAPTURE
HOOGSTEEN BASEPAIRING
REVERSE HOOGSTEEN BASEPAIRING

FIG. 13